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CANCER EPIDEMIOLOGY

Serologic markers of *Chlamydia trachomatis* and other sexually transmitted infections and subsequent ovarian cancer risk: Results from the EPIC cohort

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Abbreviations: CI, confidence interval; EOC, epithelial ovarian cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; FTP, full-term pregnancy; HGSC, high-grade serous ovarian; HPV, human papillomavirus; HSV-2, herpes simplex virus type 2; MFI, median fluorescence intensity; MHT, menopausal hormone therapy; OC, oral contraceptive; PID, pelvic inflammatory disease; RR, relative risk; STI, sexually transmitted infections.

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Abstract

A substantial proportion of epithelial ovarian cancer (EOC) arises in the fallopian tube and other epithelia of the upper genital tract; these epithelia may incur damage and neoplastic transformation after sexually transmitted infections (STI) and pelvic inflammatory disease. We investigated the hypothesis that past STI infection, particularly *Chlamydia trachomatis*, is associated with higher EOC risk in a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort including 791 cases and 1669 matched controls. Serum antibodies against *C. trachomatis*, *Mycoplasma genitalium*, herpes simplex virus type 2 (HSV-2) and human papillomavirus (HPV) 16, 18 and 45 were assessed using multiplex fluorescent bead-based serology. Conditional logistic regression was used to estimate relative risks (RR) and 95% confidence intervals (CI) comparing women with positive vs. negative serology. A total of 40% of the study population was seropositive to at least one STI. Positive serology to *C. trachomatis* Pgp3 antibodies was not associated with EOC risk overall, but with higher risk of the mucinous histotype (RR = 2.30 [95% CI = 1.22-4.32]). Positive serology for chlamydia heat shock protein 60 (cHSP60-1) was associated with higher risk of EOC overall (1.36 [1.13-1.64]) and with the serous subtype (1.44 [1.12-1.85]). None of the other evaluated STIs were associated with EOC risk overall; however, HSV-2 was associated with higher risk of endometrioid EOC (2.35 [1.24-4.43]). The findings of our study suggest a potential role of *C. trachomatis* in the carcinogenesis of serous and mucinous EOC, while HSV-2 might promote the development of endometrioid disease.

KEYWORDS

Chlamydia trachomatis, herpes simplex virus, human papillomavirus, *Mycoplasma genitalium*, ovarian cancer

1 | INTRODUCTION

Epithelial ovarian cancer (EOC) is a heterogeneous disease, with distinct histologic subtypes hypothesized to arise via different pathways of carcinogenesis.[1] The low-grade serous, endometrioid and clear cell histotypes have generally accepted origins and/or precursor lesions,[2,3] and are suggested to be more strongly associated with well-known EOC risk factors (eg, oral contraceptive [OC] use, parity, number of life-time ovulations, tubal ligation, hysterectomy and endometriosis), while risk factors for high-grade serous ovarian cancer (HGSC), the most common and lethal subtype, are less well defined and risk factor associations are generally weaker for HGSC than for other subtypes.[4] Evidence is accumulating that the majority of ovarian cancers originate in non-ovarian epithelial tissue, for example, the distal fallopian tubes (HGSC)[1,5] or ectopic endometrial tissue (endometrioid/clear cell).

Given the likely extra-ovarian origin of a proportion of EOCs, exposures associated with tubal pathologies are of increasing interest with respect to EOC risk. Sexually transmitted infections (STIs) are associated with a range of gynecologic sequelae including pelvic inflammatory disease (PID),[6,7] PID has been associated with EOC risk,[8,9] though data to date suggest strongest associations with borderline tumors.[10] *Chlamydia trachomatis* and *Mycoplasma genitalium* are two sexually transmitted bacterial causes of PID. *C. trachomatis* infects the secretory cells of the fallopian tubes in experimental animal models,[11,12] and may promote tumorigenesis by accelerating cell proliferation, inhibiting cell apoptosis (eg, via chlamydial heat shock protein 60 [cHSP60] production), promoting host DNA damage, and inducing chronic inflammation.[13] *M. genitalium* has been shown to induce chromosomal aberrations and polysomy in benign human prostate cells, and may promote anchorage-independent growth, allowing cells to detach from the surrounding extracellular matrix and metastasize, indicating possible pro-carcinogenic properties.[14] Herpes simplex virus type 2 (HSV-2), a mostly sexually transmitted virus, has been associated with higher risk of cervical cancer.[15] Finally, sexually transmitted human papillomavirus (HPV) has a well-characterized role in carcinogenesis. HPV infection has its greatest impact in the transformation zone of the uterine cervix but is also implicated in the development of anorectal carcinomas originating in, or close to, the anorectal squamocolumnar epithelial junction.[16] Similarly, the distal end of the fallopian tubes harbors the potentially vulnerable junction of the fallopian tubal epithelium and the peritoneal mesothelium.[17]

Epidemiologic data on STIs and EOC risk are limited and the results divergent,[18-25] and few studies are prospective.[18,22,25,26] The largest and most recent prospective study (n = 337 cases) observed a twofold higher risk of EOC among women seropositive to chlamydial Pgp3 antibodies,[25] with similar associations between relatively high circulating antibodies in another recent prospective study[18]; no associations were observed for *M. genitalium*, the investigated HPV types (L1 proteins of types 16, 18 and 45), or HSV-2.

Based on experimental and epidemiologic data we hypothesized that STIs, and *C. trachomatis* in particular, may play a role in the development of EOC. Given the few prospective studies to date, and no studies by EOC histotype beyond serous vs nonserous disease, the aim of our study was to assess the association between STI serostatus, analyzed in prospectively collected blood samples, and EOC risk, overall and by histologic subtype, in a case-control study nested in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

What's new?

Sexually transmitted infections (STI) have been linked with pelvic inflammatory disease but their association with ovarian cancer remains unclear. In this large prospective study, serum antibodies against *Chlamydia trachomatis* were associated with higher epithelial ovarian cancer risk, though some associations were limited to select histotypes. Herpes simplex virus type 2 infection was associated with endometrioid ovarian cancer, a rarer ovarian cancer subtype. These findings underscore that STIs may be important in the etiology of ovarian cancer and may represent a target for primary prevention.

2 | MATERIALS AND METHODS

2.1 | Study population: The EPIC cohort

EPIC is an ongoing prospective cohort including 521 330 participants (367 903 women) selected from the general population generally aged 25-70 years, and enrolled from 1992 to 2000 in 23 centers across 10 European countries: Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. Study design, population and data collection have been described previously in detail.[27] Briefly, information on lifestyle, diet, reproductive and anthropometric factors was collected at baseline. A total of 226 673 women provided a blood sample at recruitment. Participants provided written informed consent at baseline and the Ethical Committee of IARC and the University of Heidelberg approved our study.

2.2 | Nested case-control study participant selection

Study design and case and control selection of this nested case-control study have been described previously.[28] Briefly, cases of epithelial ovarian, fallopian tube and primary peritoneal cancer were identified through linkages with cancer registries, health insurance records, and direct contact with cohort members. Data on histologic subtype and tumor grade were obtained from pathology reports and cancer registries.

Up to four controls per case were randomly selected using incidence density sampling among all women from the cohort having a

blood sample, with no reported oophorectomy, and alive and free of cancer at the time of diagnosis of the index case. Cases and controls were matched on study recruitment center, age at blood donation, time of the day of blood collection, fasting status, exogenous hormone use at blood donation as well as menstrual cycle phase for premenopausal women. Further details are provided in the Supplemental Methods. A total of 791 cases and 1669 controls were included in the study (85 cases with 1 control; 606 cases with 2 controls, 8 cases with 3 controls and 82 cases with 4 controls).

2.3 | Laboratory assays

Prediagnosis levels of antibodies to *C. trachomatis*, *M. genitalium*, HSV-2 and HPV were measured using multiplex fluorescent bead-based serology assays and quantified as median fluorescence intensity (MFI) levels.[29] Samples from cases and controls were analyzed within the same analytical batch and laboratory personnel were blinded to case-control status. *C. trachomatis* infection history was assessed measuring antibodies to Pgp3[18] and cHSP60-1, plus MOMP-D, MOMP-A, MOMP-L2, TARP-F2 and TARP-F1 from serovar D.[30] MOMPs representing all three biovars were included given the high cross-reactivity between MOMP serovars, and the low prevalence of trachoma (serovar A-C) in Western European countries. Antibodies to Pgp3, a chlamydia plasmid-encoded protein, sometimes referred to as the "gold standard" marker of current or previous infection,[18,31,32] as well as antibodies to cHSP60-1, produced with persistent *C. trachomatis* infection,[33] were the primary *C. trachomatis* antibodies of interest. The Pgp3 antibody was positive in 79.5% of women reporting *C. trachomatis* infection in a recent study,[31] and cHSP60-1 antibodies have previously been linked to tubal damage.[33] History of *M. genitalium* was assessed using MgPa N-Terminus, and rMgPa antibodies.[18] HSV-2 antibodies to 2mgG unique, specific for HSV-2,[34] were assessed. HPV infection was determined using antibodies to types 16, 18 and 45 oncoproteins E6 and E7 and the major capsid protein L1.[29] An STI was defined as seropositive according to cut-off values and rules summarized in Table S1.

2.4 | Statistical analyses

Conditional logistic regression was used to calculate odds ratios, as estimates of relative risks (RRs), and 95% confidence intervals (95% CIs) comparing participants seropositive to those seronegative for the individual infections using the laboratory cut-off levels. Further, a recent study observed significant associations between *C. trachomatis* and EOC in one population using the laboratory cut-off, but in another population only after applying a higher cut-off level to define *C. trachomatis* seropositivity using the Pgp3 antibody.[18] Therefore, we also evaluated associations between the individual *C. trachomatis* antibodies and EOC risk comparing "low positive" (laboratory cut-off < $MFI_{individual}$ < median in positive women) and "high positive" ($MFI_{individual}$ ≥ median in positive women), to seronegative. History of infection with different STIs could potentially lead to worse tissue damage, and a tendency toward higher risk of EOC with antibodies to *C. trachomatis* plus a second infection was found in one study.[25] Thus, infection with *C. trachomatis* plus any other infection (*M. genitalium*, HSV-2, HPV), relative to women negative to all infections, was investigated. The following were evaluated as potential confounders/covariates: ever menopausal hormone therapy (MHT) use (never, ever), number of full-term pregnancies (FTP; continuous), duration of OC use (continuous) and smoking status (never, former, current; further evaluated as never, former quit 20+ years, former quit 11-20 years, former quit ≤10 years, current occasional, current 1-15 cigarettes/day, current 16-25 cigarettes/day, current 26 cigarettes/day). Final multivariable models include duration of OC use and number of FTPs. Missing values (OC duration, 3%; number FTPs, 10.6%) were imputed to the most frequently observed value for that variable (OC use = never, FTP = 2); results were unchanged when restricted to women with data on OC use and parity. RRs changed <10% after including the remaining variables.

We investigated STIs and EOC overall, by tumor histology (serous, mucinous, endometrioid, clear cell and NOS), and for HGSC disease (tumor grades 2 or 3). Heterogeneity in the associations between the STIs and EOC risk by disease subtype was assessed comparing models assuming the same association across subtypes to a model assuming different associations across subtypes using the likelihood ratio test.[35] We conducted a sensitivity analysis restricted to parous women. Associations between STIs and EOC risk were evaluated by age at blood donation (<60, ≥60 years), lag time between blood donation and diagnosis (<5, ≥5 years), OC use (ever, never), menopausal status at blood collection (premenopausal, perimenopausal and postmenopausal); the Wald test was used to assess heterogeneity in associations.

Statistical analyses were conducted using SAS software, version 9.3 (SAS Institute, Cary, NC). *P* values are two-sided and $P < .05$ was considered statistically significant.

3 | RESULTS

Median age at blood collection was 56.5 years (range = 29.9-80.7) and the majority of cases and controls were postmenopausal at blood collection (cases, 69.1%; controls, 69.6%) and reported at least one full-term pregnancy (cases, 83.1%; controls, 88.8%; Table 1). Cases were diagnosed at median age 62.9 years (range = 30.6-86.5), a median of 6.3 years (range = 0.04-16.0) after blood collection (Table 2). The majority of cases were of serous histology (54.7%) followed by "not otherwise specified" (16.7%), endometrioid (11.8%), mucinous (7.3%) and clear cell (4.7%). Of the 464 cases with grade data (59% of all cases, 66% of serous cases), 90.5% had moderately or poorly differentiated tumors. A total of 40% of the study population (39.5% of controls; 41.0% of cases) was seropositive for at least one of the investigated STIs. Seroprevalence varied by study country, with the highest prevalence of antibodies to the evaluated STIs generally observed in the Nordic countries (eg, ≥42.3% positive for

TABLE 1 Baseline characteristics of EOC cases and matched controls: EPIC ovarian cancer nested case-control study

	Cases (n = 791)	Controls (n = 1669)
Age at blood donation (years)[a]	56.5 (29.9, 80.7)	56.5 (30.1, 79.3)
Age at menopause (years)[b]	50.0 (32.0, 63.0)	50.0 (30.0, 63.0)
Menopausal status[a]		
Premenopausal	132 (16.7)	280 (16.8)
Perimenopausal	112 (14.2)	227 (13.6)
Postmenopausal	547 (69.1)	1162 (69.6)
Age at menarche (years)[b]	13.0 (9.0, 20.0)	13.0 (8.0, 20.0)
Age at first delivery (years)[b]	24.0 (16.0, 40.0)	24.0 (14.0, 45.0)
Ever full term pregnancy[b]	604 (83.1)	1358 (88.8)
Number of full-term births[b]		
None	123 (17.3)	172 (11.6)
1	113 (15.9)	247 (16.6)
2	288 (40.5)	632 (42.5)
3	116 (16.3)	284 (19.1)
4+	72 (10.1)	151 (10.2)
Ever OC use[b]	331 (43.7)	812 (50.7)
Duration of OC use (years)[b]	5.00 (1.0, 25.0)	5.00 (1.0, 25.0)
Ever MHT use[b]	229 (32.0)	488 (32.6)
Duration of MHT use (years)[b]	4.0 (0.1, 27.0)	3.0 (0.1, 20.0)
BMI (kg/m^2)	25.2 (17.2, 45.4)	25.0 (15.5, 50.6)
Smoking[b]		
Never	421 (54.2)	932 (56.9)
Former	181 (23.3)	377 (23.0)
Current	174 (22.4)	329 (20.1)

Note: Values are shown as median (range) or number (percentage).
[a]Matching factor.
[b]Number of missing values (cases, controls): age at menopause among postmenopausal women (101, 208), age at menarche (39, 82), age at first delivery (4, 4), ever full-term pregnancy (64, 139), number of full-term pregnancies (79, 183), OC use (33, 68), duration of OC use (20, 55), MHT use (75, 170), duration of MHT use (33, 61) and smoking (15, 31).
Abbreviations: BMI, body mass index; EOC, epithelial ovarian cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; MHT, menopausal hormone therapy; OC, oral contraceptive.

TABLE 2 Characteristics of EOC cases: EPIC ovarian cancer nested case-control study

	EOC cases (n = 791)
Age at diagnosis (years)	62.9 (30.6, 86.5)
Histology	
Serous[a]	433 (54.7)
Mucinous	58 (7.3)
Endometrioid	93 (11.8)
Clear cell	37 (4.7)
NOS	132 (16.7)
Others	38 (4.8)
Stage[b,c]	
Local	113 (16.3)
Regional	124 (17.8)
Distant metastatic	458 (65.9)
Grade[c]	
Well differentiated	44 (9.5)
Moderately differentiated	162 (34.9)
Poorly or undifferentiated	258 (55.6)
Time between blood collection and EOC diagnosis (years)	6.3 (0.04, 16.0)
Time between blood collection and EOC diagnosis	
<5 years	304 (38.4)
≥5 years	487 (61.6)

Note: Values are shown as median (range) or number (percentage).
[a]*n* = 268 high-grade serous (data on grade available for 66% [n = 284] of serous cases).
[b]Local: stage I, regional: stage II and IIIa, distant metastatic: stage >IIIb.
[c]Number of missing cases: Stage 96, grade 327.
Abbreviations: EOC, epithelial ovarian cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; NOS, not otherwise specified.

C. trachomatis in Sweden, Denmark and Norway vs 15.1% positive in Spain; Table S2).

Seropositivity to *C. trachomatis* Pgp3 antibodies was not associated with EOC risk overall (Table 3). Suggestive heterogeneity by histologic subtype was observed (P = .07 using lab cutoff; P = .01 using median MFI in seropositive cutoff). Seropositivity to Pgp3 was associated with a 2.3-fold higher risk of mucinous EOC (RR = 2.30 [95% CI = 1.22-4.32]); this association was robust to adjustment for smoking (RR = 2.49 [1.29-4.79]) (result not tabled). No significant associations were observed for other histotypes and no clear patterns emerged in analyses evaluating "high positive" (above the median among women seropositive) and "low positive" (below the median among women seropositive) vs seronegative. Women seropositive for the cHSP60-1 antibody had 36% higher risk of EOC overall (1.36 [1.13-1.64]), and 44% higher risk of serous disease (1.44 [1.12-1.85]), relative to seronegative women; as with the Pgp3 antibodies, there was no pattern in analyses by high and low seropositive vs seronegative.

We observed associations between the other investigated *C. trachomatis* antibodies and EOC risk. Positive MOMP-A serology was significantly associated with higher risk of overall EOC using the laboratory cut-off (1.25 [1.04-1.50]), and "high positive" serology for MOMP-A and MOMP-D were associated with higher risk of serous EOC (eg, MOMP-D, 1.44 [1.07-1.92]; Table S3). Relatively high levels of TARP-F1 antibodies were significantly associated with mucinous EOC (2.29 [1.09-4.78]). Distributions of cases and controls by seropositivity using the laboratory cut-off as well as positives above and below median antibody level are provided in Table S4.

M. genitalium, HSV-2 and HPV16 E6, or HPV18 E6 + E7, or HPV45 E6 + E7 were not associated with EOC risk, except a positive

TABLE 3 Seropositivity to *Chlamydia trachomatis* and EOC risk overall and by histological subtypes; EPIC ovarian cancer nested case-control study

	C. trachomatis (Pgp3)			*C. trachomatis* (cHSP60-1)		
	Controls *n* (%)	Cases *n* (%)	RR[a] (95% CI)	Controls *n* (%)	Cases *n* (%)	RR[a] (95% CI)
EOC						
Negative	1176 (70.5)	547 (69.2)	ref	1426 (85.4)	647 (81.8)	ref
+, lab cut-off	493 (29.5)	244 (30.8)	1.05 (0.89-1.24)	243 (14.6)	144 (18.2)	1.36 (1.13-1.64)
+, MFI < median[b]	245 (14.7)	123 (15.5)	1.05 (0.85-1.29)	124 (7.4)	69 (8.7)	1.29 (1.00-1.66)
+, MFI ≥ median[b]	248 (14.9)	121 (15.3)	1.05 (0.85-1.30)	119 (7.1)	75 (9.5)	1.43 (1.11-1.85)
Serous						
Negative	616 (67.8)	302 (69.7)	ref	772 (84.9)	348 (80.4)	ref
+, lab cut-off	293 (32.2)	131 (30.3)	0.88 (0.71-1.09)	137 (15.1)	85 (19.6)	1.44 (1.12-1.85)
+, MFI < median[b]	158 (17.4)	63 (14.5)	0.77 (0.58-1.02)	73 (8.0)	38 (8.8)	1.19 (0.85-1.67)
+, MFI ≥ median[b]	135 (14.9)	68 (15.7)	1.02 (0.77-1.35)	64 (7.0)	47 (10.9)	1.71 (1.22-2.40)
High-grade serous[c]						
Negative	404 (69.8)	197 (73.5)	ref	482 (83.2)	223 (83.2)	ref
+, lab cut-off	175 (30.2)	71 (26.5)	0.82 (0.63-1.08)	97 (16.8)	45 (16.8)	1.01 (0.73-1.40)
+, MFI < median[b]	90 (15.5)	37 (13.8)	0.82 (0.57, 1.19)	54 (9.3)	16 (6.0)	0.66 (0.40-1.07)
+, MFI ≥ median[b]	85 (14.7)	34 (12.7)	0.83 (0.57, 1.20)	43 (7.4)	29 (10.8)	1.45 (0.96-2.19)
Mucinous						
Negative	93 (73.8)	32 (55.2)	ref	110 (87.3)	47 (81.0)	ref
+, lab cut-off	33 (26.2)	26 (44.8)	2.30 (1.22-4.32)	16 (12.7)	11 (19.0)	1.83 (0.91-3.65)
+, MFI < median[b]	13 (10.3)	11 (19.0)	2.20 (0.97-5.00)	6 (4.8)	4 (6.9)	1.87 (0.66-5.30)
+, MFI ≥ median[b]	20 (15.9)	15 (25.9)	2.36 (1.17-4.75)	10 (7.9)	7 (12.1)	1.80 (0.75-4.31)
Endometrioid						
Negative	135 (68.2)	62 (66.7)	ref	162 (81.8)	75 (80.6)	ref
+, lab cut-off	63 (31.8)	31 (33.3)	1.04 (0.66-1.64)	36 (18.2)	18 (19.4)	1.11 (0.66-1.89)
+, MFI < median[b]	24 (12.1)	16 (17.2)	1.50 (0.82-2.76)	18 (9.1)	10 (10.8)	1.32 (0.66-2.62)
+, MFI ≥ median[b]	39 (19.7)	15 (16.1)	0.78 (0.44-1.41)	18 (9.1)	8 (8.6)	0.93 (0.46-1.88)
Clear cell						
Negative	49 (69.0)	28 (75.7)	ref	60 (84.5)	34 (91.9)	ref
+, lab cut-off	22 (31.0)	9 (24.3)	0.70 (0.32-1.58)	11 (15.5)	3 (8.1)	0.57 (0.16-2.00)
+, MFI < median[b]	12 (16.9)	9 (24.3)	1.75 (0.74-4.15)	5 (7.0)	3 (8.1)	1.59 (0.50-5.08)
+, MFI ≥ median[b]	10 (14.1)	0	—[d]	6 (8.5)	0 (0)	—[d]
NOS						
Negative	219 (78.5)	93 (70.5)	ref	247 (88.5)	110 (83.3)	ref
+, lab cut-off	60 (21.5)	39 (29.5)	1.62 (1.06-2.48)	32 (11.5)	22 (16.7)	1.56 (0.96-2.52)
+, MFI < median[b]	26 (9.3)	18 (13.6)	1.66 (0.99-2.80)	15 (5.4)	11 (8.3)	1.85 (0.94-3.66)
+, MFI ≥ median[b]	34 (12.2)	21 (15.9)	1.58 (0.92-2.72)	17 (6.1)	11 (8.3)	1.35 (0.70-2.60)
P_{het} by subtype (lab cut-off)			.07			.68
P_{het} by subtype (MFI </≥ median)			.01			.36

[a]RRs estimated from ORs from a conditional logistic regression model; Cases and controls were matched on study recruitment center, age at blood donation (±6 months), time of the day of blood collection (±1 hour), fasting status (<3, 3-6, >6 hours); exogenous hormone use at blood donation (no/yes) as well as menstrual cycle phase for premenopausal women ("early follicular" (days 0-7 of the cycle), "late follicular" (days 8-11), "periovulatory" (days 12-16), "mid-luteal" (days 20-24), "other luteal" (days 17-19 or days 25-40), or missing). Adjusted for number of full term pregnancies and duration of use of oral contraceptives (years); results for mucinous disease robust to additional adjustment for smoking.

[b]+, MFI < median indicates positive serology using lab cut-off, and MFI value below median among women seropositive according to lab cut-off ("low positive"); +, MFI ≥ median indicates positive serology using lab cutoff, and MFI value above median among women seropositive according to lab cut-off ("high positive").

[c]Data on grade available for 66% of serous cases.

[d]Not estimable or not reliable.

Abbreviations: CI, confidence interval; EOC, epithelial ovarian cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; NOS, not otherwise specified; P_{het}, heterogeneity by subtype assessed with the likelihood ratio test; RR, relative risk.

TABLE 4 Seropositivity to STIs and EOC risk overall and by histological subtypes; EPIC ovarian cancer nested case-control study

	Mycoplasma genitalium			HSV-2			HPV16 E6, or HPV18 E6 + E7 or HPV45 E6 + E7[b]		
	Controls *n* (%)	Cases *n* (%)	RR[a] (95% CI)	Controls *n* (%)	Cases *n* (%)	RR[a] (95% CI)	Controls *n* (%)	Cases *n* (%)	RR[a] (95% CI)
EOC									
Negative	1552 (93.0)	738 (93.3)	ref	1484 (88.9)	706 (89.3)	ref	1627 (97.5)	775 (98)	ref
Positive	117 (7.0)	53 (6.7)	0.93 (0.70-1.23)	185 (11.1)	85 (10.7)	0.95 (0.75-1.19)	42 (2.5)	16 (2)	0.78 (0.48-1.26)
Serous									
Negative	847 (93.2)	404 (93.3)	ref	807 (88.8)	388 (89.6)	ref	885 (97.4)	426 (98.4)	ref
Positive	62 (6.8)	29 (6.7)	0.95 (0.66-1.37)	102 (11.2)	45 (10.4)	0.89 (0.66-1.21)	24 (2.6)	7 (1.6)	0.61 (0.30-1.24)
Mucinous									
Negative	115 (91.3)	55 (94.8)	ref	109 (86.5)	53 (91.4)	ref	120 (95.2)	58 (100)	
Positive	11 (8.7)	3 (5.2)	0.52 (0.15-1.82)	17 (13.5)	5 (8.6)	0.57 (0.23-1.40)	6 (4.8)	0	—[c]
Endometrioid									
Negative	181 (91.4)	82 (88.2)	ref	182 (91.9)	77 (82.8)	ref	196 (99.0)	91 (97.8)	ref
Positive	17 (8.6)	11 (11.8)	1.33 (0.71-2.48)	16 (8.1)	16 (17.2)	2.35 (1.24-4.43)	2 (1.0)	2 (2.2)	2.14 (0.40-11.44)
Clear cell									
Negative	60 (84.5)	36 (97.3)	ref	58 (81.7)	34 (91.9)	ref	69 (97.2)	35 (94.6)	
Positive	11 (15.5)	1 (2.7)	—[c]	13 (18.3)	3 (8.1)	0.33 (0.08-1.41)	2 (2.8)	2 (5.4)	2.53 (0.38-16.70)
NOS									
Negative	265 (95.0)	125 (94.7)	ref	251 (90)	118 (89.4)	ref	275 (98.6)	127 (96.2)	ref
Positive	14 (5.0)	7 (5.3)	1.20 (0.53-2.74)	28 (10)	14 (10.6)	1.13 (0.62-2.06)	4 (1.4)	5 (3.8)	2.25 (0.77-6.55)
P_{het} by subtype			.05			.08			.05

[a]RRs estimated from ORs from a conditional logistic regression model; Cases and controls were matched on study recruitment center, age at blood donation (±6 months), time of the day of blood collection (±1 hour), fasting status (<3, 3-6, >6 hours); exogenous hormone use at blood donation (no/yes) as well as menstrual cycle phase for premenopausal women ("early follicular" (days 0-7 of the cycle), "late follicular" (days 8-11), "periovulatory" (days 12-16), "midluteal" (days 20-24), "other luteal" (days 17-19 or days 25-40), or missing). Adjusted for number of full term pregnancies and duration of use of oral contraceptives (years).

[b]Results for HPV16, 18 or 45 L1: EOC (RR: 0.87 [0.66-1.14]), serous (RR: 0.95 [0.66-1.36]), mucinous (RR: 0.42 [0.14-1.28]), Endometrioid (RR: 1.05 [0.48-2.30]), Clear cell (RR: 0.39 [0.07-2.32]), NOS (RR: 1.21 [0.64-2.28]), P_{het} by subtype = 0.59.

[c]Not estimable or not reliable.

Abbreviations: CI, confidence interval; EOC, epithelial ovarian cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; HPV, human papillomavirus; HSV-2, herpes simplex virus type 2; NOS, not otherwise specified; P_{het}: heterogeneity by subtype assessed with the likelihood ratio test; RR, relative risk; STI, sexually transmitted infection.

association between HSV-2 and endometrioid EOC (2.35 [1.24-4.43]; P_{het} by histotype = 0.08; Table 4). No associations were observed for the HPV-related markers stated above; results were similar when the L1 antibodies were evaluated (Table 4, footnote). Seropositivity to *C. trachomatis* plus a second STI was not associated with EOC risk in any subgroup (data not shown).

3.1.1. | Sensitivity and subgroup analyses

Results were not materially different in sensitivity analyses restricted to parous women (data not shown). We observed limited heterogeneity in associations by age at blood collection (<60 vs ≥60 years; Table S5). In the analysis considering "high" and "low" positive antibody levels to *C. trachomatis* cHSP60-1 antibodies and EOC risk, women with high positive antibody levels had higher risk of EOC only among women <60 at blood collection (P_{het} = 0.04; <60 years, 1.91 [1.31-2.78]; ≥60 years, RR: 0.89 [0.49-1.62]). Significant heterogeneity by age at blood collection was also observed for the association between HSV-2 and EOC risk (P_{het} = 0.01; <60 years, 0.59 [0.48-1.00]; ≥60 years, 1.51 [0.97-2.34]). No heterogeneity in associations was observed by lag time (<5 years, ≥5 years) or OC use (ever, never; data not shown); however a borderline significant association between positive Pgp3 serology and EOC risk was observed in women diagnosed within 5 years of blood collection (1.32 [0.97-1.80]), but not in women diagnosed ≥5 years from blood collection (0.90 [0.69-1.16]; P_{het} = .06). No significant heterogeneity in associations was observed by menopausal status at blood collection (premenopausal, perimenopausal, postmenopausal; P_{het} > .19); however, a statistically significant positive association between cHSP60-1 and EOC risk was only observed among women postmenopausal at blood collection (2.05 [1.15-3.64]).

4 | DISCUSSION

Individual *C. trachomatis* antibodies were associated with higher risk of EOC, in particular, cHSP60 antibodies were associated with EOC overall and the serous subtype, while Pgp3 was associated with the mucinous subtype, in this large, prospective study. Furthermore, we observed an association between HSV-2 and the endometrioid EOC subtype. *M. genitalium* and HPV showed generally null associations with EOC risk, and we observed no associations between the investigated STIs and HGSC.

Our findings of positive associations between select *C. trachomatis* antibodies and EOC risk are in line with the results of three previous studies[18,19,25]; however, others have observed no association.[20,22,24] Previous studies have predominantly analyzed ovarian cancer as a single disease, with small sample size precluding analyses by subtype beyond serous vs nonserous disease.[18-20,22,24,25] In the current study, we provide a detailed investigation by histotype, observing suggestive heterogeneity by tumor histology. Pgp3 serology indicating current or past infection with *C. trachomatis* was not associated with EOC risk overall in contrast to two recent studies,[18,25] but was associated with significantly higher risk of mucinous EOC; this has not previously been described. This result was robust to statistical adjustment for smoking, in addition to OC use and parity; however, the number of mucinous ovarian cancer cases was limited (n = 58) and this result should be interpreted with caution.

Positive serology for cHSP60-1 antibodies was associated with higher risk of EOC overall, and serous disease, as were relatively high levels of the MOMP-A, MOMP-D and MOMP-L2 antibodies. cHSP60-1 IgG antibodies were associated with Type II EOC in a subgroup analysis in a previous study,[22] and cHSP60-1 antibodies were associated with EOC using higher cut-off levels in a retrospective case-control, but not the prospective component of the study by Trabert et al.[18] Chlamydial HSP60 is produced by the chlamydia bacteria to induce a persistent state of infection in the host cell, thereby escaping immune defense.[36] This leads to inhibited cell apoptosis increasing the risk for a DNA-damaged cell to survive, and being further exposed to an inflammatory environment with cytotoxic substances. This state of persistent chlamydia infection with increased cHSP60 production is one pathogenic mechanism by which chlamydia could cause ovarian cancer.[37] Given that cHSP60 is associated with persistent chlamydia infection, cHSP60-1 antibodies may be a marker of persistent cHSP60-producing *C. trachomatis* infection, thus positive serology for cHSP60-1 antibodies might show an association with ovarian cancer even in the absence of an association with Pgp3 antibodies, a more general marker of infection. The explanation for differences in associations observed between the European population in the current study and the two prospective studies in U.S.-based populations is not immediately evident. One explanation may be regional differences in *C. trachomatis* strains,[38] with different strains possibly having different downstream impacts on the genital tract epithelium; studies characterizing the impact of different strains of *C. trachomatis* on the genital tract are required to clarify potential differing sequelae by strain. Further, differences in patterns of seeking healthcare, and differences in screening, detection and treatment of STIs, may account for the differences in associations in the study populations.

M. genitalium is a small intracellular bacterium known to cause PID. Serum antibodies to *M. genitalium* were not associated with EOC overall or any histotypes in our study, in line with the findings of prospective studies,[18,22] although an association was reported in a retrospective case-control study population[18] and parous women in another study.[25]

Positive HSV-2 serostatus was associated with a higher risk of endometrioid EOC. A higher, but not statistically significant, risk of EOC overall was also found in the recent study by Trabert et al.[18] Women with positive serology for HSV-2 had higher risk of endometrial (uterine) cancer in the NHANES (National Health and Nutrition Examination Survey).[15] Ovarian cancer of the endometrioid subtype shares several characteristics with endometrial cancer and is synchronous with endometrioid cancer of the uterus in 15%-20% of cases.[39] Similar to previous studies that found no association of HPV serostatus with ovarian cancer,[18,25,26,38] no associations were observed in our study.

The hypothesis that HGSC would be the ovarian cancer subgroup most likely associated with STI serum markers was not supported by the results of our study, though these analyses were limited by the availability of data on grade (available for 59% of all cases, 66% of serous cases) and, thus, a limited number of HGSC cases (n = 268). One explanation for the lack of association observed may have been due to limited power to detect an association in this subgroup. A possible biologic interpretation is that the secretory cells of the distal fallopian tube might not be vulnerable to neoplastic transformation caused by infectious agents, even though one recent study suggests that *C. trachomatis* has long-term impact on the tubal epithelium by altering the phenotype and inducing heritable changes in the epigenome.[40] Only one prospective seroepidemiological study has analyzed the association of STI antibodies and Type II EOC finding a significant association of *C. trachomatis* antibodies with Type II.[22] Another explanation could be that analyses of serum antibodies do not reflect the previous infections that are crucial in malignant transformation of the secretory cells.

Our study has several strengths. First, the present study included the largest number of cases among seroepidemiological studies focusing on STI antibodies and risk of EOC published to date, allowing a more detailed evaluation by histological subtypes and grade than previous studies. Second, the prospective design excluded disease-related effects on serologic antibody levels; however, no data are available on the timing of infection prior to the blood collection, nor infections in the interval between blood collection and diagnosis/selection as a control. Third, a validated multiplex Luminex assay allows us to evaluate antibodies to several candidate STIs in the study. Seroconversion appears to occur in relatively close temporal proximity to diagnosis, with a total of 92% of women with a positive Pgp3 serology at chlamydia diagnosis and ≥1 day to 6 months after diagnosis, and positive serology was observed in 72% of individuals >4 years after diagnosis in one study.[41] Wills et al[42] reported the sensitivity of a Pgp3 ELISA at 73.8% among women with known prior chlamydia diagnoses and ≥96% specificity. The Pgp3 assay used in the current study has excellent agreement with ELISA (kappa ≥ 94%[18]). A limitation of our study, despite the large number of cases overall, is that only about two-thirds of serous cases had histological grade, which limited the analyses of the HGSC subgroup. Case numbers in other subgroups were limited as well. The analyses were adjusted for the known risk (or protective) factors parity and OC use. Other known risk factors not adjusted for due to lack or limitations of available data included hysterectomy, tubal ligation, endometriosis and family history of ovarian cancer; thus, residual confounding cannot fully be excluded. However, adjustment for family history of ovarian and/or breast cancer had minimal impact on effect estimates in the NHS/NHSII study, and restriction to participants without tubal ligation in that study resulted in findings similar to the overall results.[25] Many statistical tests are reported; therefore, some significant associations may be due to chance and the results have to be interpreted with caution.

In conclusion, our study supports a possible role of *C. trachomatis* and HSV-2 in ovarian carcinogenesis. History of STIs might be of importance in the etiology of serous, mucinous and endometrioid ovarian cancer. The results of our study need to be confirmed in other prospective cohorts of sufficient size to investigate STIs and risk by tumor histotype. Experimental studies delineating the mechanisms linking STIs to EOC, and the primary prevention potential of STI prevention, are tasks for future experimental, translational and epidemiological research to resolve.

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CONFLICT OF INTEREST

No potential conflicts of interest were disclosed.

DATA ACCESSIBILITY

For information on how to submit an application for gaining access to EPIC data, please follow the instructions at http://epic.iarc.fr/access/index.php.

DISCLAIMER

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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